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A limited number of reprints of publications are available.
Those not available are marked with an asterick (*).

Information (mimeographed):

27 Separation of diastase and protein from wheat through the action of sulphites. September, 1943.

Journal articles:

A. K. Balls and S. R. Hoover. The milk-clotting action of papain. Jour. Biol. Chem. 121:737-745. November, 1937.
A new method for the estimation of papain is given in detail, based on its property of clotting milk. The method is much simpler and quicker than any others proposed, even yet. The kinetics involved are also discussed. The paper recounts experiments on the mechanism of the activation of papain by phenylhydrazine in which it is shown that this reagent accelerates milk-clotting like hydrogen sulphide and cysteine. The milk-clotting property appears to be a function of the proteinase present not of the action of more or less hypothetical peptidases. Measurements of the temperature optimum of papain made by the new technique are given.

W. S. Hale. The proteinase in wheat flour. Cereal Chem. 16:695-702. September, 1939.
A proteinase was extracted from patent flour with 10 percent sodium chloride solution. This extract was purified by fractional precipitation with ammonium sulphate. The enzyme was purified further by dialysis to remove the ammonium sulphate. A reference preparation was then made for comparison with proteinases previously prepared from wheat bran and whole wheat. Examination of its behavior toward oxidizing agents and reducing agents led to the conclusion that it is an enzyme of the papain type. This flour proteinase was found to be activated by cysteine and inactivated by iodoacetic acid and a variety of bread improvers. There was no evidence that this proteinase from patent flour is different from that extracted from wheat bran or whole wheat flour.

*A. K. Balls and H. Lineweaver. Isolation and properties of crystalline papain. Jour. Biol. Chem. 130:669-686. October, 1939.
This paper gives the methods for preparing crystals of a proteolytic protein from papaya latex. This protein was named papain by the authors. Two crystal habits are described, needles and six-sided plates. The molecular weight and some other analytical data on the protein are given. The ability of the pure protein to clot milk, hydrolyze proteins, and split hippurylamide (a peptidase-like function) is shown. Mixed crystals of active and inactive enzyme protein were also obtained.

A. K. Balls and F. E. Arana. Recent observations on the curing of vanilla beans in Puerto Rico. Proc. 8th Amer. Sci. Cong. 7:187-191. 1940.
Experiments on the respiration rate of green vanilla beans showed that the respiratory processes were faster immediately after injury to the tissue. Methods of curing vanilla intentionally produce such injuries, and may be useful in speeding up oxidation. The role of peroxidase in the chemistry of vanilla curing is discussed.

A. K. Balls and W. S. Hale. A sulphur-bearing constituent of the petroleum ether extract of wheat flour. Cereal Chem. 17:243-245. March, 1940.
Patent flour was extracted with petroleum ether, the extract concentrated and chilled and the insoluble material separated. The remaining extract was then mixed with three volumes of 1 N HCl in alcohol, whereupon a precipitate was formed. The precipitate was then washed with absolute alcohol and ether and dried in vacuo. This material was no longer soluble in petroleum ether, but was soluble in water and dilute alcohol. The water solution after reduction gave a powerful nitroprusside test. Analyses showed 13.4 per cent total nitrogen, 3.9 per cent protein nitrogen, 2.94 percent sulfur, and no phosphorous. Probably the extractable flour lipids contain a substance bearing a reversibly oxidizable SH group that could act as an activator for the flour proteinase.

A. K. Balls and W. S. Hale. The effect of ethylene on freshly harvested wheat. Cereal Chem. 17:490-494. July, 1940.
Freshly combined harvested wheat when treated with small concentrations of ethylene (1-10,000) showed marked improvement in its bread-making qualities. An immediate increase in the germinating ability of the wheat was noted after it was treated with a low concentration of ethylene, but there is evidence that higher concentrations cause a decidedly harmful effect. Results of these experiments show the possibility of using ethylene to hasten the well-known after-ripening process, popularly designated as "sweating".

*A. K. Balls, R. R. Thompson, and W. W. Jones. Crude papain. Indus. and Eng. Chem. 32:1144-1147. August, 1940.
New sources of papain were investigated. The green fruit of the papaya appears to contain very little enzyme after the latex has been removed by tapping. The pressed juice from the leaves and stems of the papaya plant contain considerable papain. By acidifying and filtering this juice, it becomes stable and can be used as a source of enzyme preparations.

Papain is precipitated by alcohol or ammonium sulphate. Both of these methods yield a product similar to commercial papain. Yield studies showed that from a ton of fresh plant material, 12 pounds of papain could be recovered.

*A. K. Balls, H. Lineweaver, and S. Schwimmer. Drying of papaya latex -- stability of papain. Indus. and Eng. Chem. 32:1277-1279. September, 1940.
Reasons for the instability of commercial papain have been sought. Deterioration is rapid during and after drying of the latex and is faster in air than in vacuum. Part of the inactivated enzyme is reactivated by cyanide. More activity is lost when the latex is diluted prior to use. The latex is shown to contain a thermostable oxidation factor that destroys the enzyme. Methods for minimizing these losses are suggested. Stable enzyme preparations are obtained by adding salt to the latex and partially drying. Loss on dilution is minimized by diluting with boiled distilled water.

*H. Lineweaver and S. R. Hoover. A comparison of the action of crystalline papain on native and urea denatured proteins. Jour. Biol. Chem. 137:325-335. January, 1941.
Data indicate that papain is probably able to attack certain native proteins, although slowly. The increased rate of digestion shown by denatured proteins parallels the increase in the chemical reactivity of certain groups, namely, S-S, -SH, and phenolic OH. These results appear to be unaltered by the mode of denaturation employed.

A. K. Balls, R. R. Thomspon, and M. W. Kies. Bromelin, Properties and commercial production. Indus. and Eng. Chem. 33:950-953. July, 1941.
The fate of bromelin, the proteolytic enzyme of pineapple juice, has been followed throughout the factory operations of pineapple canning. The enzyme appears in the juice and is there remarkably resistant to heat. A method is suggested which laboratory experiments (only) have indicated might be used to recover bromelin from low grade juice without decreasing the yield of alcohol. The curious behavior of bromelin toward heating and alkali has been studied, and it was found that the activity of a bromelin preparation at a fixed pH depended upon the previous pH at which the preparation was held. The facts appear to be best explained by the assumption that the enzyme protein easily undergoes reversible denaturation.

A. K. Balls, W. S. Hale, and T. H. Harris. A crystalline-protein obtained from a lipoprotein of wheat flour. Cereal Chem. 19:279-288. March, 1942.
A crystalline protein-like material was prepared from the petroleum ether extract of patent flour. Analyses show that the principal amino acid residues are arginine and cystine. The crystals are the hydrochloride of a basic substance resembling the protamines. This hydrochloride has a minimum molecular weight of 6000 and a probable molecular weight of double that value. It therefore lies on a borderline between proteins and polypeptides. In the flour the material exists in the reduced form and is probably the activator of the wheat proteinase. It is thought that this protein-like material exists in the flour combined with a phosphorous-bearing lipid, and is therefore a component of one number of the little-understood class of lipoproteins.

E. J. Coulson, T. H. Harris and B. Axelrod. Effect on small laboratory animals of the injection of the crystalline hydrochloride of a sulphur protein from wheat flour. Cereal Chem. 19:301-307. March, 1942.
A crystalline sulphydryl compound of protein-like nature (purothionin) obtained from wheat was investigated pharmacologically. The LD_{50} for guinea pigs on intravenous injection was approximately 1.6 mg./kilo. The MLD by the intraperitoneal route for mice was of the same order of magnitude as for rabbits, i.e., 12-15 mg./kilo. Rabbits were barely affected by 13.8 mg./kilo. On intravenous injection the LD_{50} for guinea pigs was 1.6 mg./kilo. Rabbits appeared to be somewhat more resistant. Guinea pigs tolerated the oral administration of 50-100 lethal intravenous doses. Five-hour acid hydrolysis greatly reduced the toxicity of the compound, judging by the effects of intraperitoneal injection into mice. Concentration of the protein as low as 1 part in 1,250,000 caused the contraction of the isolated guinea pig uterine strip. The response to this substance, unlike histamine, depended upon the presence of calcium ion within very narrow limits of concentration. Acid hydrolysis of the protein completely destroyed its uterus-contracting property.

*L. S. Stuart and T. H. Harris. Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. Cereal Chem. 19:288-300. March, 1942.
Crystalline purothionin, obtained from wheat flour, was found to be antibiotic against certain bacteria and fungi, particularly saphrophytic types. The potency of purothionin as a "germicide" is considerable, but the substance is also quite toxic. See Coulson, Harris and Axelrod, Cereal Chem. 19:301. 1942.

A. K. Balls. A crystalline sulphur-protein from wheat. Jour. Wash. Acad. Sci. 32:132-137. May, 1942.
An article summarizing the work to date on purothionin, the crystalline protein obtained by the Enzyme Laboratory from wheat. Its preparation and partial composition are given, and the reactions of this protein as part of an oxidation-reduction system are discussed. The effect of purothionin on the activity of crystalline chymopapain and on the oxidation of fats by lipoxidase from soybean is particularly stressed.

A. K. Balls and F. E. Arana. The curing of vanilla. Indus. and Eng. Chem. 33:1073-1075. August, 1941: Revista de Agricultura, Industria & Commercio de Puerto Rico 34(20) 167-172. 1942.
The complete oxidation of aromatic substances during the curing of vanilla may lead to loss of aroma. On the theory that intermediate, not final, products of oxidation are desired, the beans were frozen. This reduces their CO_2 output during curing, and apparently allows enzymes of the peroxidase class an opportunity to store up intermediate oxidation products. The practical results of this modification of the curing process have been very satisfactory. See U. S. Patent #2,274,120 to Balls and Arana.

M. W. Kies and S. Schwimmer. Observations on proteinase in brain. Jour. Biol. Chem. 145:685. October, 1942.
Brain proteinase has been partially purified and shown to be of the catheptic type. Di- and tripeptidases were found to be present in brain tissue. No evidence was obtained for the presence of a lipolytic enzyme similar to the one previously reported in muscle. The results indicate the presence of a surprisingly large amount of cathepsin in brain as compared with muscle. Brain, however, autolyzes neither more rapidly nor to a greater extent than muscle under similar conditions. While the effect of the brain cathepsin undoubtedly contributes to the difficulty of handling brain tissue commercially, it does not seem to afford adequate explanation of the rapid disintegration of the material that is so frequently observed in the packing industry.

A. K. Balls, W. S. Hale and T. H. Harris. Further observations on a crystalline wheat protein. Cereal Chem. 19:840-844. November, 1942.
A new crystalline protein isolated from patent flour is described. The crystals are the hydrochloride of a substance that appears to be related to the protamines. It is rich in arginine and cystine, and appears to be made up entirely of amino acid residues. Purothionin is suggested as a name for this protein material. Further information as to the construction of this protein was obtained by digestion with various crystalline and purified proteolytic enzymes. The extent of digestion was measured by the Van Slyke Apparatus. Evidence is given showing that at least one half of the nitrogen linkages in purothionin are the same as those occurring in proteins, and that the amino acids are linked in chains of 6 or 8 (probably 6).

A. K. Balls and I. W. Tucker. Extraction of diastase and recovery of protein from wheat. Fruit Prod. Jour. 23(1):15. 1943.
A description of the activation and solution of beta-amylase from wheat flour by dilute sulphite solutions, and the further use of the otherwise wasted wheat enzyme to fortify malt in the ordinary conversion process. During the extraction much of the gluten present forms a clot that may be easily removed and dried.

A. K. Balls. Control of enzymatic action in foods. Proc. Inst. Food Tech. pp. 165-169. 1943.
Besides a general discussion of the problem of enzyme action in foods, this paper gives a full account of our experiments on the inactivation of peroxidase by heat and its subsequent return to activity on standing. The matter was then important because the use of peroxidase tests for blanching was required by the Army, as well as because of the information on the composition and action of peroxidase that these experiments contributed. The work was continued in a paper by Schwimmer, Jour. Biol. Chem. 154, 361 (1944) q.v.

W. S. Hale, S. Schwimmer, and E. G. Bayfield. Studies on treating wheat with ethylene. I. Effect upon high-moisture wheat. Cereal Chem. 20(2): 224-233. March, 1943.
The application of ethylene gas in the proportion of 1 part of ethylene to 100,000 parts of air to freshly harvested, high-moisture wheat in semi-commercial bins increased the respiration. The treated wheat did

not heat as rapidly or as much as the untreated. The grade of the grain, percentage germination, and baking performance of the treated samples were superior to the untreated during several months of storage. The experiments indicate that ethylene gas does not prevent spoilage of high-moisture wheat on storage, but the heating of such wheat may be materially retarded, possibly because of an artificial ripening of the greener kernels present.

*A. K. Balls, B. Axelrod and M. W. Kies. Soybean lipoxidase. Jour. Biol. Chem. 149(2):491-504. August, 1943.
Soybean lipoxidase, sometimes referred to as carotene oxidase, has been purified considerably. An extremely simple assay method based on the rate of oxidation of carotene in the presence of ethyl linoleate has been devised. Study of the purified enzyme led to the discovery of a heat-stable polypeptide material in soybean extracts which markedly enhances the catalytic effect of the enzyme. The activator appears to exert its effect by combining with the fat rather than with the enzyme. The inhibition of lipoxidase by purothionin, a polypeptide found in wheat, may also be caused by its effect on the fat emulsion. The substrate specificity of lipoxidase has been investigated with pure fatty acids. Linoleic, linolenic, and arachidonic acids were the only ones oxidized by the enzyme of considerable number of acids tested. These acids are the so-called nutritionally essential fatty acids and it has been suggested that their function in the animal may be linked with a similar oxidative reaction.

A. K. Balls. Desmo enzymes. Vortex 4(10):338-340, 342-344. December, 1943.
A summary of our work up to that time on the amylase of starch. This paper contains a description of the liberation of beta-amylase from wheat by sulphite solutions, and gives data which indicate that the enzyme exists in the flour as an inactive but activable "zymogen" protein. The properties of the zymogen are such that it comes very close to Willstätter's conception of a desmo enzyme. Possibly many desmo enzymes require chemical alteration (activation) as well as physical solution before they can be demonstrated.

G. Y. Gottschall. The activation of papain during digestion of meat. Food Res. 9:6-10. 1944.
Partially inactive papain becomes more active during the digestion of some proteins, but not of others. The progressive activation was shown to be due to the reducing action of sulphydryl groups uncovered during the proteolysis. The initial degree of activation required for papain digestion thus depends upon the nature of the substrate.

S. Schwimmer. Comparison of crude and purified preparations of a leucyl-peptidase associated with beef muscle. Jour. Biol. Chem. 154(2):361. July, 1944.
A highly active specific enzyme capable of hydrolyzing leucylglycine and leucyldiglycine but not simple glycine or alanine peptides, has been found associated with beef tissue. Its properties have been compared with a crude glycerol extract of beef muscle. The properties investigated (pH vs. activity, specificity, activation by manganese, stability) indicate that the purified enzyme is a leucyl-peptidase, whereas the starting material contained more than one peptide-splitting enzyme.

S. Schwimmer. Regeneration of heat inactivated peroxidase. Jour. Biol. Chem. 154(2):487. July, 1944.
The regeneration as a function of time and temperature was studied. It was found that regeneration is largely a function of the heating rate, has a positive temperature coefficient, and is a time reaction. Factors that are essential for regeneration exist in both the precipitate formed upon heating and in the supernatant therefrom. Evidence is given to show that vegetables may contain more than one peroxidase, and that these enzymes vary in their activities toward iodide and pyrogallol, respectively. A vegetable may be characterized by comparing the iodide and pyrogallol oxidizing activities. The precipitate formed when vegetable juice is heated carries these characteristic properties of the peroxidase with it. Reappearance of the enzyme after heat treatment involves resolution of this insoluble component, recombination with a soluble group, which may well be the same for the vegetables studied, and reversion of enzyme protein to its native state.

A. K. Balls and S. Schwimmer. Digestion of raw starch. Jour. Biol. Chem. 156(1):203. November, 1944.
Uncooked starch granules may readily be broken down (as observed microscopically) and digested to maltose and glucose by a mixture of hog pancreas and Aspergillus oryzae. A necessary inorganic factor in the ash of wheat flour was found to be calcium. The pH and temperature optima are 5.2 and 55° C., respectively. Different starches vary in the readiness with which they are broken down. Potato starch is relatively resistant compared to corn and wheat starches. The size of the starch granule does not seem to be a determining factor in this resistance.

S. Schwimmer. The role of maltase in the enzymolysis of raw starch. Jour. Biol. Chem. 161(1):219. November, 1945.
The enzymolysis of raw starch by mixtures of Aspergillus oryzae and pancreatic amylase has been studied in detail and compared with their action on cooked starch. The complementary action has been traced to the maltase of the Aspergillus. The observations made have been interpreted to mean that the maltase decreases the operation of the following factors which tend to present complete conversion by the alpha-amylase; irreversible inactivation; reversible inhibition by maltose, resynthesis from maltose; slow rate of hydrolysis of the "abnormal" linkages present in amylopectin. These interfering factors can be equally minimized in the absence of maltase by concomitant dialysis of the amylase-starch reaction mixture. Consequently, raw starch can be completely digested by pancreatic amylase alone in a dialysis apparatus. Whereas the difference in the action of alpha-amylase on raw and cooked starch seems to be one of rate imposed upon the system by limited substrate available, the complete lack of susceptibility of the starch granule to attack by beta-amylase has been attributed to the masking (by strong hydrogen bonding) of the non-reducing end of the glucose chains.

I. W. Tucker. Effect of flour lipids on recovery of gluten from hard and soft wheat flours by the use of sulfite solutions. Cereal Chem. 23:2. March, 1946.
The recovery of gluten from flour by coagulating it with sulphite solutions (apparently due to activation of a proteolytic enzyme) is fairly complete with the high-protein (hard wheat) flour but very small with low-protein

(soft wheat) flour. If the lipids are extracted from soft wheat, results approaching those with hard-wheat flours are obtained. Conversely, if flour lipids are added to hard-wheat flour, the recovery of gluten is low, as with soft-wheat flour. Apparently the ratio of protein to lipid determines the ease of coagulation of the gluten, and a mechanical explanation is suggested, based on the assumption that a heavy film of fat on the surface of a gluten particle prevents its coalescence with other coated particles, and thus prevents the formation of a clot.

A. K. Balls, R. R. Thomspon and M. K. Walden. A crystalline protein with beta-amylase activity, prepared from sweet potatoes. Jour. Biol. Chem. 163(2). May, 1946.
Preparation of crystalline beta-amylase from sweet potatoes is given, together with data characterizing the substance as a protein.

A. K. Balls, Enzyme actions and food quality. Food Tech. 1(2):245. 1947.
Enzyme action continues in fruits, vegetables and grains after harvest. The ultimate quality of these food products depends a great deal on how they are treated after harvesting. Enzyme action can be stopped by heat or it can be slowed by refrigeration. The choice of one or the other for each type of food determines to a great extent the final quality of the food product. The enzyme system responsible for ripening of fruits is very important and the proper control of these enzymes makes a great deal of difference in the ultimate quality of the food product.

B. Axelrod. Citrus fruit phosphatase. Jour. Biol. Chem. 167:57-72. January, 1947.
Citrus fruits contain an enzyme system capable of hydrolyzing a variety of phosphomonoesters. Relative concentration of the enzyme in various tissues has been determined. The enzyme of the Navel orange juice (first reported instance of an enzyme in citrus juice) has been concentrated and the following characteristics studied: specificity, pH optimum, stability to pH, energy of activation, and Michaelis constant for digestion of nitrophenyl phosphate.

B. Axelrod. Phosphatase activity as an index of pasteurization in citrus juices. Fruit Prod. Jour. and Amer. Food Manufacturer 26:132-133. January, 1947.
Effects of temperature, duration of heating, and pH on the destruction of acid phosphomonoesterase of citrus fruits have been determined, in order to evaluate the use of phosphatase activity as an index of pasteurization of citrus products. A positive phosphatase test on freshly pasteurized orange and grapefruit juice shows that much less than the customary degree of pasteurization has been used. It is possible to determine by this test whether frozen citrus products have or have not been pasteurized.

S. Schwimmer. Sources of beta-amylase as supplements to barley malts in saccharification and fermentation. Cereal Chem. 24:70-78. January, 1947.
Within the limits of concentration wherein the rate of reaction is proportional to the enzyme concentration, the calculated saccharifying power of malt and flour mixtures are about the same as the experimentally

determined activity. The latter value tends to be higher than that calculated for mixtures of sweet potato and malts high in alpha-amylase activity. When mixtures of malts and supplement are used in fermentation tests in equi-amylolytic amount, the subsequent yield of alcohol is greater for sweet potato supplemented mashes. These results are consistent with the demonstration of sugar-changing but non-amylolytic enzyme in sweet potatoes.

S. Schwimmer. Development and solubility of amylase in wheat kernels throughout growth and ripening. Cereal Chem. 24:167-178. May, 1947.
The rate of development of the beta-amylase of the active growing wheat kernel is five times that of dry weight increase. The apparent decrease in beta-amylase upon ripening has been shown to be due to increasing insolubility, which is probably a consequence of the change in character of the wheat proteins during the dehydration of the wheat. These solubility relationships in the mature kernel do not change with artificial moisture changes. The beta-amylase content of the average size kernels is directly proportional to their weight. Alpha-amylase is present in about the same amount per kernel through growth and development.

W. G. Rose. Synthesis of cephalin. Jour. of the Amer. Chem. Soc. 69:1384-1387. June, 1947.
Two new methods are described for the synthesis of cephalin. The amino group of ethanolamine was protected by a phthalyl group or a carbobenzoxy group, the protected ethanolamine was allowed to react with dipalmitoglycerophosphoryl chloride, and the protecting group was then cleaved with hydrazine or phosphonium iodide, respectively. The dipalmitocephalin so obtained melted at 195-198° with decomposition.

M. K. Kies. Activation of soybean lipoxidase. Jour. Biol. Chem. 170:121-132. September, 1947.
A crystalline polypeptide which enhances the oxidation of carotene-ethyl linoleate by lipoxidase has been isolated from soybeans, but is apparently different from the amorphous material previously described. An activator has been demonstrated in gum arabic. Preliminary experiments suggest that this activator is concerned with the formation of a compound from linoleic acid which absorbs in the region of 280 mμ. It is tentatively suggested from the present data that the "activator" acts on the substrate rather than on the enzyme and that in so doing it affects either the formation or the utilization of fat peroxide.

S. Schwimmer. Purification of barley malt alpha-amylase. Cereal Chem. 24:315-325. September, 1947.
Purification studies on the alpha-amylase of malt extracts have led to preparations, 1 mg. of which is as active as 5 grams of malt. The purification procedure, which included heating, salt fractionation and bentonite absorption, was elaborated by following the protein nitrogen as well as the activity of the possible purification steps. The stability and hydrolytic properties of the purified alpha-amylase are in agreement with these properties generally attributed to alpha-amylase.

PATENTS

*A. K. Balls and W. S. Hale. Method for the softening of dough. U. S. Patent 2,103,443. December 28, 1937.

*T. L. Swenson. Process for reducing foam of fermented egg white to liquid albumin. U. S. Patent 2,110,613. March 8, 1938.

*A. K. Balls, H. Lineweaver and S. Schwimmer. Processes for the preparation of papain. U. S. Patent 2,257,218. September 30, 1941.

*A. K. Balls, A. G. Kevorkian, and F. E. Arana. Process for curing vanilla beans. U. S. Patent 2,274,120. February 24, 1942.

*A. K. Balls and W. S. Hale. Sulphydryl compound obtained from flour. U. S. Patent 2,313,504. March 9, 1943.

*A. K. Balls and E. F. Jansen. Proteolytic enzyme process. U. S. Patent 2,313,875. March 16, 1944.

*A. K. Balls and W. S. Hale and T. H. Harris. Proteinous material. U. S. Patent 2,366,952. January 9, 1945.

*A. K. Balls and W. S. Hale. Method of treating cereal grains. U. S. Patent 2,381,421. August 7, 1945.